Andrologia

WILEY

RESEARCH ARTICLE OPEN ACCESS

Protective Effects of the Aqueous Extract of *Tribulus cistoides* (Zygophyllaceae) Against Nicotine-Induced Fertility Impairment in Male Wistar Rats

Kiafon Betrand Nsah | Nguedem Sylvie Fonkwo | Njoya Moses Tita Mogho | Tangu Patience Neng | Mumbi Laurantine Ngenteh | Ndifor Rose Nchang | Bih Belta Lilian Fubi | Kada Sanda Antoine

Department of Zoology, Faculty of Science, University of Bamenda, P.O. Box 39, Bambili, Cameroon

Correspondence: Kada Sanda Antoine (antoinekada@gmail.com)

Received: 30 August 2025 | **Revised:** 11 March 2026 | **Accepted:** 18 March 2026

Academic Editors: Harleen Khatra and R. Henkel

Keywords: male infertility | nicotine | oxidative stress | testosterone | *Tribulus cistoides*

ABSTRACT

Infertility affects a substantial proportion of couples worldwide and is defined as the failure to achieve pregnancy after 1 year of regular, unprotected sexual intercourse. Growing interest has been directed toward ethnobotanical approaches for the management of male infertility. This study evaluated the therapeutic potential of the aqueous extract of *Tribulus cistoides* against nicotine-induced infertility in male Wistar rats. Thirty adult male rats were randomly allocated into six groups ($n = 5$). Group 1 received distilled water (5 mL/kg), Group 2 received nicotine alone (1 mg/kg), and Group 3 received nicotine plus testosterone enanthate (5 mg/kg), while Groups 4–6 were administered nicotine in combination with *T. cistoides* aqueous extract at doses of 50, 100, and 150 mg/kg, respectively, for a period of 60 days. Evaluated parameters included body and reproductive organ weights, testicular oxidative stress markers, seminal vesicle fructose, total testicular protein, sperm density and motility, serum testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testicular histoarchitecture. Nicotine administration resulted in a significant reduction in sperm density ($15.00 \pm 1.05 \times 10^6$/g vs. $43.05 \pm 3.06 \times 10^6$/g), sperm motility ($35.73\% \pm 0.72\%$ vs. $56.44\% \pm 2.17\%$), and serum testosterone levels (0.124 ± 0.002 ng/mL vs. 0.521 ± 0.048 ng/mL) compared with the normal control group. Conversely, treatment with *T. cistoides* extract at 150 mg/kg significantly improved sperm density ($50.15 \pm 1.46 \times 10^6$/g), motility ($71.16\% \pm 4.15\%$), and testosterone levels (0.411 ± 0.016 ng/mL). In contrast, testosterone enanthate did not produce significant improvements in the fertility parameters assessed when compared with the nicotine-treated group. The extract also enhanced testicular antioxidant status, reproductive hormone profiles, seminal fructose levels, testicular protein content, and reproductive organ weights, likely due to its androgenic and antioxidant properties. Histological examination further revealed increased sperm density in the extract-treated groups like in the normal control group. In conclusion, the aqueous extract of *T. cistoides* exhibited notable protective and restorative effects against nicotine-induced male infertility, highlighting its potential as a natural therapeutic agent for improving male reproductive function.

1 | Introduction

Infertility is defined as the inability of a couple to conceive after 12 months of regular, unprotected sexual intercourse. Globally, infertility affects at least one in five couples [1], and it is categorized into primary infertility (no prior successful pregnancy) and secondary infertility (following at least one previous live birth). In Africa, both types are nearly equally prevalent, reported at 49.91% and 49.79%, respectively [2]. Male factors contribute

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Copyright © 2026 Kiafon Betrand Nsah et al. *Andrologia* published by John Wiley & Sons Ltd.

significantly to infertility, accounting for ~25.6% of cases [3]. In Cameroon, male infertility particularly primary infertility is notably widespread [4]. In men, infertility is frequently linked to abnormalities in semen quality or quantity, including conditions such as oligospermia (low sperm count), asthenozoospermia (reduced sperm motility), azoospermia (absence of sperm in the ejaculate), and teratospermia (abnormally shaped sperm) [2, 5]. The underlying causes are diverse and multifactorial, encompassing urogenital tract infections, sexual dysfunction, and the use of substances such as cannabinoids, opioids, and psychotropic drugs, which may suppress gonadotropin-releasing hormone (GnRH) secretion [6]. Other contributors include congenital malformations, acquired urogenital abnormalities, bilateral obstructions, and endocrinological disorders such as congenital GnRH deficiencies. Environmental exposures further exacerbate the problem, as pesticides, insecticides, alcohol, nicotine, and other chemical pollutants adversely affect male fertility by disrupting sperm production and function [6]. Some of these factors lead to hormonal imbalances in men, ultimately resulting in reduced androgen levels, which are a major cause of male infertility [7]. Nicotine, in particular, poses a significant threat with an estimated 19% of adults globally using tobacco and up to 33% among men. Tobacco use remains a serious public health concern [8]. According to the World Health Organization, over 8 million deaths annually are attributed to tobacco, including 1.3 million from secondhand smoke exposure [9]. Nicotine, which constitutes 90%–95% of the alkaloids in cigarettes, is rapidly absorbed via the respiratory tract, oral mucosa, and skin, with most of it metabolized in the liver [10]. It adversely affects the male reproductive system, significantly reducing sperm motility, count, and density in a dose-dependent manner. Nicotine has been reported to cause a decrease in male reproductive hormones such as testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) [10]. Laboratory studies further reveal that even short-term exposure to nicotine impairs sperm viability and motility, underscoring its potential to harm fertility beyond habitual smoking [11].

The management of male infertility generally targets the underlying etiology and may involve pharmacological interventions such as testosterone therapy, estrogen synthesis inhibitors, antiestrogens, and phosphodiesterase-5 (PDE5) inhibitors including sildenafil, vardenafil, and tadalafil. Although these treatments can be effective, they are often associated with adverse effects such as headaches, hypertension, gastrointestinal disturbances, and anxiety [12]. The growing demand for safer, more accessible, and cost-effective alternatives has therefore renewed interest in traditional medicine and plant-based therapies. In recent years, several medicinal plants have been experimentally evaluated for their potential fertility-enhancing effects in male Wistar rats.

For instance, *Tribulus terrestris* has been reported to improve sexual behavior and libido in sexually sluggish male albino rats [13]. Similarly, the methanolic extract of *Momordica foetida* (Cucurbitaceae) was shown to protect against Parastar-induced reproductive dysfunction and testicular oxidative stress in male rats [14], while the hydroethanolic extract of *Rauvolfia vomitoria* demonstrated improvements in testicular antioxidant status and testosterone levels in male Wistar rats [15]. Within the genus Tribulus, *T. terrestris* remains the most extensively studied species with respect to male reproductive function. The evaluation of its aphrodisiac effect in sexually sluggish male rats by Singh et al. [13] provided important preliminary evidence; however, the experimental model presents certain limitations, particularly the lack of clear justification for the induction or characterization of sexual sluggishness. In contrast, the present study employed nicotine-induced infertility, a well-documented and reproducible model of male reproductive dysfunction, thereby providing a more clearly defined pathological framework. In another study, the impact of *T. terrestris* on testicular morphology and sperm production in male rats was investigated [16]. Although treatment for 40 days improved testicular histoarchitecture, no significant enhancement in sperm production was observed. Notably, a 40-day treatment period does not fully encompass the spermatogenic cycle in rats (~54 days), which may explain the absence of significant changes in sperm density. To address this limitation, our study extended treatment to 60 days to ensure full coverage of the spermatogenic cycle and allow adequate time for detecting changes in spermatogenic output. Similarly, *Tribulus alatus* has been reported to possess fertility-enhancing properties following 30 days of treatment in male rats [17]. However, this duration is shorter than the complete spermatogenic cycle, potentially limiting the interpretation of long-term reproductive outcomes. Despite the considerable interest in medicinal plants for the management of male infertility and the extensive investigation of certain species within the Tribulus genus, *Tribulus cistoides* has not been scientifically evaluated for its potential effects on male reproductive function. This absence of experimental data constitutes a significant knowledge gap within the genus. Given the pharmacological relevance of related Tribulus species and the methodological limitations observed in prior studies, investigating *T. cistoides* under a well-characterized infertility model and over a complete spermatogenic cycle is both scientifically justified and necessary. *T. cistoides*, commonly known as puncture vine, is a member of the Zygophyllaceae family. Alongside *T. terrestris* and *T. alatus*, it has been traditionally used to support male reproductive health. *T. cistoides* is an annual plant native to tropical regions of Africa, Asia, and the Americas [18]. In Cameroon, particularly in the Far North Region, the plant is widely used in traditional medicine for the treatment of male infertility, where it is most commonly prepared as a decoction by boiling the plant material in water. The use of an aqueous extract in the present study was therefore chosen to replicate this traditional mode of preparation, ensuring ethnopharmacological relevance and translational applicability of the findings. In addition, *T. cistoides* has been reported to be used in the management of urogenital disorders, including kidney and bladder dysfunctions, in other parts of the world [19, 20]. Based on these traditional uses, the present study aimed to evaluate the effects of an aqueous extract of *T. cistoides* on nicotine-induced male infertility in Wistar rats.

2 | Materials and Methods

2.1 | Plant Collection and Authentication

In February 2024, *T. cistoides* whole plant was collected from the Logone-et-Chari Division, located in the Far North Region of Cameroon at coordinates 12° 04′ 60″ N, 14° 49′ 60″ E. The plant was subsequently taken to the Cameroon National Herbarium for identification. Its authenticity was confirmed by comparing it with a reference specimen (specimen no. 67586/HNC) previously collected by Moussa and preserved in the herbarium. After collection, the plant material was thoroughly rinsed with tap water

and air-dried at room temperature for 1 week. Once dried, it was ground into fine powder using a Binatone electric blender. The powdered material was then sieved to obtain a uniform consistency, which was used to prepare the aqueous extract.

2.2 | Preparation of the Aqueous Extracts

Following the method described by Zhang et al. [21], the aqueous extract was prepared using the decoction technique. Specifically, 200 g of the powdered plant material was soaked in 2000 mL of distilled water and heated at 100°C for 20 min. After cooling to room temperature, the mixture was filtered through Whatman No. 1 filter paper. The resulting filtrate was then concentrated to dryness in a thermostat oven (Model: DHG-9101-1 SA) set at 45°C to obtain the crude aqueous extract. The dried extract was weighed using an electronic balance (Model: JJ500), yielding 33.06 g equivalent to a 13.224% extraction yield. The final product was carefully scraped, transferred into a tightly sealed, labeled container, and stored in a refrigerator for later experimental use.

2.3 | Experimental Animals

In this study, 30 male albino Wistar rats (*Rattus norvegicus*), aged 2 months and weighing between 100 and 150 g, were obtained from a breeder in Bamenda, located in the Northwest Region of Cameroon. The animals were then transported to the animal facility of the Department of Zoology at the Faculty of Science, University of Bamenda, where they underwent an acclimatization for a period of 2 weeks before the start of the experiment. All procedures were conducted in accordance with the ethical guidelines set by the Cameroon National Veterinary Laboratory and adhered to the principles of the 1964 Declaration of Helsinki and its subsequent amendments. Ethical approval for the study was granted by the Cameroon National Veterinary Ethics Committee (Approval and Health Control Testimonial No. 003/19CCS/MINEPIA/RD-NW/DDME/SSV) [22]. Throughout acclimatization and the experimental period, the rats were maintained under standard laboratory conditions: temperature between 24°C and 28°C, relative humidity of 60%–70%, and a 12-h light/dark cycle. They had unrestricted access to food and water.

2.4 | Animal Grouping and Treatment

The previously acclimatized animals were randomly assigned into six groups, with five rats per group. Treatments were administered orally once daily for a period of 60 days except for testosterone which was administered intramuscularly once a week. The animals were treated as follows:

- Group 1: Received distilled water (5 mL/kg) and served as the normal control.
- Group 2: Received nicotine at a dose of 1 mg/kg body weight (negative control).
- Group 3: Received testosterone enanthate at 5 mg/kg body weight (positive control).
- Groups 4, 5, and 6: Received *T. cistoides* aqueous extract at doses of 50 mg/kg, 100 mg/kg, and 150 mg/kg of body weight, respectively, in combination with nicotine at 1 mg/kg.

The treatment duration was established based on the protocol described by Amira et al. [23], which evaluated the effects of *Panax ginseng* on male fertility in cypermethrin-exposed rats, and was further justified by the duration of the complete spermatogenic cycle in rats, which takes 2 months. The dose of nicotine was selected in accordance with a previously validated experimental model of nicotine-induced reproductive toxicity [24]. The dose of testosterone enanthate used as the positive control was chosen based on the study by Tangu et al. [22], which demonstrated the protective effects of an aqueous extract of *Ganoderma boninense* (Ganodermataceae) against hepatic and reproductive alterations in Parastar-exposed male Wistar rats, using testosterone as a reference treatment. The doses of *T. cistoides* aqueous extract (50, 100, and 150 mg/kg body weight) were determined based on the study by Singh et al. [13], who evaluated the aphrodisiac activity of *T. terrestris* in sexually sluggish male albino rats using comparable dose ranges. Although the two species are distinct, they belong to the same genus (Tribulus) and are reported to share similar classes of bioactive phytoconstituents, notably steroidal saponins and flavonoids. In the absence of prior dose–response data specific to *T. cistoides*, these doses were considered appropriate to investigate potential biological effects while ensuring experimental safety. Throughout the 8-week treatment period, the body weight of each rat was recorded weekly using an electronic balance (Model: JJ500). At the end of the experimental period, the animals were fasted overnight on day 60 and euthanized on day 61 for subsequent analyses.

2.5 | Sacrifice, Collection of Blood Samples, and Reproductive Organs

At the end of the treatment period, anesthesia was induced via intraperitoneal injection of diazepam (10 mg/kg) and ketamine (5 mg/kg). The animals were then euthanized by cervical decapitation. Venous blood was collected from the jugular vein and transferred into nonheparinized tubes, where it was left to clot for 30 min. The resulting serum was separated using a micropipette, placed into cryotubes, and stored at −20°C for subsequent analysis of testosterone, FSH, and LH levels.

A meticulous dissection was then performed to extract the testes, epididymis, seminal vesicles, prostate gland, and penis. All surrounding adipose tissue was carefully removed, and the reproductive organs were weighed using an electronic balance (G and G Electronic Scale, Model: JJ500). The left testis was fixed in 10% formalin for histological analysis. The organ weight index was calculated as previously described by Mbouh et al. [14].

$$\text{Organ index} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100.$$

2.6 | Preparation of Testicular and Seminal Vesicle Homogenates

Homogenates of the testis and seminal vesicles were prepared by separately macerating each tissue in a mortar with 0.1 M phosphate buffer (pH 7.2) as earlier described by Mbouh et al. [14]. The resulting mixtures were then centrifuged at 3000 rpm for 15 min using a Hettich EBA 3S centrifuge. The supernatants were carefully collected, transferred into cryotubes, and stored at −20°C for subsequent biochemical analysis.

2.7 | Biochemical Analysis

2.7.1 | Evaluation of Testicular Oxidative Stress Biomarkers

The concentrations of key oxidative stress markers, namely, reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS), and catalase (CAT) in the testicular tissue were measured following the protocol outlined by Mbouh et al. [14]. The level of nitric oxide (NO) was assessed using the Griess assay, as described by Olasehinde et al. [25], which detects nitrite, a stable end-product of NO metabolism. Additionally, the enzymatic activity of superoxide dismutase (SOD), an important antioxidant defense enzyme, was evaluated according to the previously established method detailed in [26].

2.7.2 | Assessment of Testicular Proteins and Seminal Vesicle Fructose

The concentration of testicular proteins was determined using the Biuret method [27], which is based on the formation of a colored complex between copper ions and peptide bonds in an alkaline solution. Additionally, the level of fructose in the seminal vesicles was quantified following the procedure outlined by the World Health Organization [28], which allows for the accurate estimation of fructose, a key energy source for sperm cells.

2.7.3 | Evaluation of Serum Concentrations of Testosterone, LH, and FSH

The concentration of testosterone in the serum was measured using a quantitative enzyme-linked immunosorbent assay (ELISA) kit with a sensitivity of 0.083 ng/mL supplied by DRG International, Inc., based in Springfield, New Jersey, USA. Serum levels of FSH and LH with a detection limit of 0.8 mIU/mL were determined using quantitative ELISA kits obtained from Biorex Diagnostics, a biotechnology company located in Antrim, Northern Ireland, United Kingdom. All assays were carried out strictly according to the manufacturers' protocols as referenced in [29].

2.8 | Histological Examination of the Testis

Histopathological analysis of the testes was performed according to the method described by Goswami et al. [17]. Briefly, following sacrifice, the left testis from each animal was preserved in 10% formalin in individually labeled containers. The fixed tissues were then dehydrated through a graded ethanol series (70%, 80%, 90%, 95%, and 100%). Ethanol was subsequently cleared with two changes of xylene over a 24-h period. The tissues were infiltrated with molten paraffin wax through two changes within the same period and then embedded and blocked. Tissue sections of 5 μm thickness were obtained using a rotary microtome. A collagen solution (prepared by diluting 500 mg of collagen in 1000 mL of distilled water) was used to transfer the sections onto glass slides. Slides were deparaffinized by passing them through three changes of xylene and a descending series of graded ethanol solutions (each for 10 min), followed by rinsing in distilled water for 5 min. The sections were then stained with hematoxylin and eosin as described by Bancroft and Gamble [30]. Photomicrographs of stained tissues from both control and treated groups were captured using a light microscope (OLYMPUS BX51) at 100× magnification.

2.9 | Data Analysis

GraphPad Prism version 9 was used to analyze the data, which were then displayed for each group as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was used to compare group means. The resulting F-statistics and corresponding *p*-values are presented in the tables to indicate the overall significance of treatment effects prior to post hoc analysis. Then, the Tukey's multiple comparison test was used to examine the differences between the means of the different treatments, with a *p*-value of less than 0.05 being considered statistically significant.

3 | Results

3.1 | Effect of Aqueous Extract of *T. cistoides* on the Animal Body Weight of Male Wistar Rats Exposed to Nicotine

The body weight of animals treated with nicotine (1 mg/kg) and the doses of *T. cistoides* (50, 100, and 150 mg/kg) are presented in Figure 1. The weight of the animals increased gradually from week 1 to week 8 with no significant difference among the means at week 8 across the different experimental groups. In comparison to the negative control group, which had the lowest mean weight of 160.25 g in the group treated with nicotine (1 mg/kg of body weight), the normal control group's average weight was 165 g, increased by 3.03%. In comparison to the negative control group, the extract-treated groups experienced increases of 6.40% (50 mg/kg), 5.04% (100 mg/kg), and 4.15% (150 mg/kg) when rats were co-treated with nicotine and the various extract concentrations. In comparison to the negative control group, the testosterone treatment (5 mg/kg of body weight) resulted in a 10% gain in weight.

3.2 | *T. cistoides* Aqueous Extract's Impact on Male Wistar Rats' Reproductive Organ Weight

The weights of the testis (0.847 ± 0.060 g vs. 1.552 ± 0.089 g), epididymis (0.178 ± 0.013 g vs. 0.526 ± 0.016 g), seminal vesicle (0.131 ± 0.007 g vs. 0.294 ± 0.011 g), and prostate (0.085 ± 0.017 g vs. 0.155 ± 0.017 g) were significantly reduced in rats treated with nicotine (1 mg/kg body weight) compared with the normal control group treated with distilled water (Table 1). In contrast, the weight of the penis did not differ significantly between the nicotine-treated group and the normal control.

Compared with the negative control, co-administration of nicotine with *T. cistoides* extract resulted in a significant, dose-dependent increase in the weights of the testis (1.302 ± 0.059 g vs. 0.847 ± 0.060 g), epididymis (0.411 ± 0.037 g vs. 0.178 ± 0.013 g), and seminal vesicle (0.372 ± 0.021 g vs. 0.131 ± 0.007 g). The highest extract dose (150 mg/kg) produced the most pronounced improvement ($p < 0.001$) relative to the nicotine-only group. Additionally, combined treatment with nicotine and testosterone enanthate significantly increased the weights of the seminal vesicle (0.582 ± 0.076 g vs. 0.131 ± 0.007 g) and prostate gland (0.171 ± 0.022 g vs. 0.085 ± 0.017 g), whereas the weights of the testis and epididymis remained unchanged.

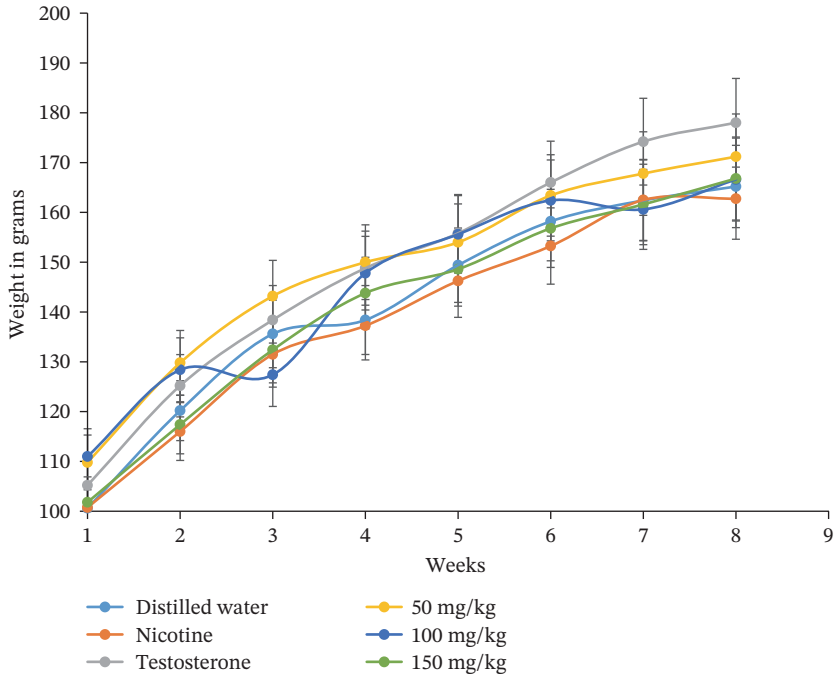


FIGURE 1 | Variation in body weight of male rats exposed to nicotine and/or aqueous extract of *T. cistoides* over a 60-day treatment period. Line graphs illustrate the different experimental groups, while dots indicate the weekly average weight for each group along the lines. Vertical bars at each point represent the standard error of the mean (SEM) for body weight.

3.3 | The Effect of Aqueous Extract of *T. cistoides* on the Sperm Density and Sperm Motility

Following the assessment of sperm motility and density, the results were summarized in Table 2. Rats treated with nicotine for 60 days showed a marked reduction in sperm density ($15.00 \pm 1.046 \times 10^6$/g of epididymis vs. $43.05 \pm 3.06 \times 10^6$/g of epididymis) and sperm motility ($35.73\% \pm 0.718\%$ vs. $56.44\% \pm 2.17\%$) compared with the normal control group treated with distilled water.

Relative to the nicotine-only (negative control) group, administration of *T. cistoides* aqueous extract significantly increased sperm density in a dose-independent manner, as all extract-treated groups exhibited comparable levels of improvement. In contrast, the effect of the extract on sperm motility was dose-dependent, with the highest dose (150 mg/kg) producing the greatest enhancement ($71.16\% \pm 4.149\%$ vs. $35.73\% \pm 0.718\%$). Conversely, treatment with testosterone enanthate did not result in a significant improvement in either sperm density or sperm motility when compared with the nicotine-treated group.

3.4 | The Effect of Aqueous Extract of *T. cistoides* on Testicular Oxidative Stress Markers of Male Wistar Rats

Table 3 presents the effects of *T. cistoides* aqueous extract on testicular oxidative stress markers. Rats treated with nicotine exhibited a marked reduction in SOD activity ($10.32\% \pm 0.651\%$I vs. $49.89\% \pm 2.279\%$I), CAT activity (3.340 ± 0.188 µmol H_2O_2 vs. 6.539 ± 0.373 µmol H_2O_2), reduced GSH levels (0.7678 ± 0.055 µM protein vs. 1.276 ± 0.036 µM protein), and NO concentration (32.39 ± 0.857 µmol/mL vs. 45.18 ± 0.844 µmol/mL), alongside a significant increase in TBARS levels (3.552 ± 0.264 nmol/mg protein vs. 1.244 ± 0.162 nmol/mg protein), compared with the normal control group treated with distilled water.

Relative to the nicotine-only (negative control) group, coadministration of *T. cistoides* aqueous extract at 150 mg/kg significantly restored antioxidant status, as evidenced by increased SOD activity ($116.1\% \pm 3.861\%$I vs. $10.32\% \pm 0.651\%$I) and CAT activity (7.389 ± 0.428 µmol H_2O_2 vs. 3.340 ± 0.188 µmol H_2O_2), as well as elevated levels of reduced GSH (1.431 ± 0.152 µM protein vs. 0.7678 ± 0.055 µM protein) and NO (55.76 ± 1.155 µmol/mL vs. 32.39 ± 0.857 µmol/mL). TBARS concentrations were also markedly reduced to near-normal values (0.913 ± 0.014 nmol/mg protein vs. 3.552 ± 0.264 nmol/mg protein).

The 100 mg/kg dose produced comparable but slightly less pronounced effects, with significant improvements observed in reduced GSH levels ($p < 0.01$) and CAT activity ($p < 0.05$) relative to the 150 mg/kg dose. Treatment with 50 mg/kg of the extract also significantly increased SOD, CAT, GSH, and NO levels while reducing TBARS concentrations when compared with the nicotine-treated group.

3.5 | The Effect of Aqueous Extract of *T. cistoides* on Testicular Total Protein and Seminal Vesicle Fructose of Male Wistar Rats Treated With Nicotine

According to the results shown in Table 4, nicotine treatment significantly decreased seminal vesicle fructose concentration (0.685 ± 0.029 mM vs. 2.483 ± 0.051 mM) compared with the normal control group treated with distilled water. Coadministration of *T. cistoides* aqueous extract at doses of 50, 100, and 150 mg/kg significantly increased seminal vesicle fructose levels in a dose-independent manner, with the 150 mg/kg dose showing the highest value (3.089 ± 0.245 mM vs. 0.685 ± 0.029 mM). This indicates that all three extract doses produced a comparable improvement in fructose concentration relative to the nicotine-only (negative control) group. Testosterone cotreatment also significantly

TABLE 1 | The effect of *T. cistoides* aqueous extract on the reproductive organs weight (g) of male Wistar rats exposed to nicotine.

Reproductive organs weight (g)	Control groups			*Tribulus cistoides* aqueous extract–treated groups			
	DW 5 mL/kg	Nicotine 1 mg/kg	TE 5 + 1 mg/kg nicotine	50 + 1 mg/kg nicotine	100 + 1 mg/kg nicotine	150 + 1 mg/kg nicotine	*F* (DFn, DFd)
Testis	1.552 ± 0.089	0.847 ± 0.060****	0.661 ± 0.041^{ns}	1.247 ± 0.071^{b}	1.265 ± 0.041^{c}	1.302 ± 0.059^{c}	F (5, 24) = 27.90
Epididymis	0.526 ± 0.016	0.178 ± 0.013****	0.258 ± 0.012^{ns}	0.428 ± 0.057^{b}	0.415 ± 0.051^{c}	0.411 ± 0.037^{c}	F (5, 24) = 15.47
Seminal versicle	0.294 ± 0.011	0.131 ± 0.007*	0.582 ± 0.076^{d}	0.311 ± 0.017^{a}	0.317 ± 0.019^{b}	0.372 ± 0.021^{c}	F (5, 24) = 18.10
Penis	0.214 ± 0.013	0.176 ± 0.007^{ns}	0.186 ± 0.011^{ns}	0.183 ± 0.018^{ns}	0.190 ± 0.010^{ns}	0.203 ± 0.015^{ns}	F (5, 24) = 1.280
Prostrate	0.155 ± 0.017	0.085 ± 0.017*	0.171 ± 0.022^{b}	0.135 ± 0.012^{ns}	0.153 ± 0.014^{ns}	0.173 ± 0.011^{b}	F (5, 24) = 4.284

Note: DW denotes distilled water (normal control), while TE denotes testosterone enanthate (positive control).
Data are expressed as mean ± SEM of organ weights, with five rats per group.
Values in the negative control group marked with superscripts (*) $p < 0.05$ and (***) $p < 0.001$ indicate statistically significant differences compared only with the normal control group.
The superscript (ns) indicates no significant difference relative to the normal control group. Values in the extract-treated groups bearing superscript letters indicate statistically significant differences compared with the nicotine-only (negative control) group: (a) $p < 0.05$, (b) $p < 0.01$, (c) $p < 0.001$, and (d) $p < 0.0001$.

TABLE 2 | The effect of *T. cistoides* aqueous extract on sperm density and motility.

Parameters	Control groups			*Tribulus cistoides* aqueous extract–treated groups			
	DW 5 mL/kg	Nicotine 1 mg/kg	TE 5 + 1 mg/kg nicotine	50 + 1 mg/kg nicotine	100 + 1 mg/kg nicotine	150 + 1 mg/kgnicotine	*F* (DFn, DFd)
Sperm density ×10^{6}/g of epididymis	43.05 ± 3.06	15.00 ± 1.046****	18.75 ± 1.186^{ns}	40.50 ± 2.640^{d}	47.15 ± 0.696^{d}	50.15 ± 1.455^{d}	F (5, 24) = 63.38
Sperm motility (%)	56.44 ± 2.17	35.73 ± 0.718***	47.18 ± 3.852^{ns}	47.35 ± 1.468^{c}	52.87 ± 1.462^{b}	71.16 ± 4.149^{d}	F (5, 24) = 20.12

Note: DW denotes distilled water (normal control), while TE denotes testosterone enanthate (positive control).
Data are presented as mean ± SEM of the evaluated parameter, with five rats per group.
Values in the negative control group marked with superscripts (***) $p < 0.001$ and (****) $p < 0.0001$ indicate statistically significant differences compared only with the normal control group. The superscript (ns) indicates no significant difference relative to the normal control group.
Values bearing superscript letters in the extract-treated groups indicate statistically significant differences when compared with the nicotine-only (negative control) group: (b) $p < 0.01$, (c) $p < 0.001$, and (d) $p < 0.0001$. Superscript (ns) indicates no significant difference relative to the nicotine-only group.

TABLE 3 | Effect of *Tribulus cistoides* aqueous extract on nicotine-induced testicular oxidative stress markers in Wistar rats.

	Control groups			*Tribulus cistoides* aqueous extract–treated groups			
Parameters	**DW 5 mL/kg**	**Nicotine 1 mg/kg**	**TE 5 + 1 mg/kg nicotine**	**50 + 1 mg/kg nicotine**	**100 + 1 mg/kg nicotine**	**150 + 1 mg/kg nicotine**	***F* (DFn, DFd)**
SOD (%I)	49.89 ± 2.279	10.32 ± 0.651****	123.9 ± 7.358^{d}	66.51 ± 3.646^{d}	93.40 ± 3.544^{d}	116.1 ± 3.861^{d}	F (5, 24) = 110.8
CAT (μmol of H_2O_2)	6.539 ± 0.373	3.340 ± 0.188****	4.798 ± 0.147^{a}	3.452 ± 0.2787^{ns}	6.898 ± 0.417^{d}	7.389 ± 0.428^{d}	F (5, 24) = 30.26
GSH (μM of protein)	1.276 ± 0.036	0.768 ± 0.055**	1.353 ± 0.121^{b}	0.7260 ± 0.065^{ns}	1.237 ± 0.033^{a}	1.431 ± 0.152^{c}	F (5, 24) = 11.81
Nitric oxide (μmol/mL)	45.18 ± 0.844	32.39 ± 0.857**	52.01 ± 4.195^{d}	32.83 ± 1.232^{ns}	46.03 ± 1.812^{b}	55.76 ± 1.155^{d}	F (5, 24) = 22.28
TBARS (nmol/mg of proteins)	1.244 ± 0.162	3.552 ± 0.264****	1.568 ± 0.163^{d}	3.142 ± 0.246^{ns}	1.769 ± 0.122^{d}	0.913 ± 0.014^{d}	F (5, 24) = 34.62

Note: DW denotes distilled water (normal control), while TE denotes testosterone enanthate (positive control).
Data are presented as mean ± SEM for each parameter, with five rats per group.
Values in the negative control group marked with superscripts (**) $p < 0.01$, (***) $p < 0.001$, and (****) $p < 0.0001$ indicate statistically significant differences compared only with the normal control group. The superscript (ns) indicates no significant difference relative to the normal control group.
Values bearing superscript letters in the extract-treated groups indicate significant differences when compared with the nicotine-only (negative control) group: (a) $p < 0.05$, (b) $p < 0.01$, (c) $p < 0.001$, and (d) $p < 0.0001$. Superscript (ns) indicates no significant difference relative to the negative control.

TABLE 4 | The effect of aqueous extract of *T. cistoides* on testicular proteins and vesicular fructose concentration.

	Control groups			*Tribulus cistoides* aqueous extract–treated groups			
Parameters	**DW 5 mL/kg**	**Nicotine 1 mg/kg**	**TE 5 + 1 mg/kg nicotine**	**50 + 1 mg/kg nicotine**	**100 + 1 mg/kg nicotine**	**150 + 1 mg/kg nicotine**	***F* (DFn, DFd)**
Seminal vesicle fructose (mM)	2.483 ± 0.051	0.685 ± 0.029****	2.580 ± 0.181^{d}	1.329 ± 0.247^{d}	2.357 ± 0.122^{d}	3.089 ± 0.245^{d}	F (5, 24) = 28.01
Testicular proteins (mg/g)	0.595 ± 0.020	0.132 ± 0.011****	0.353 ± 0.019^{d}	0.488 ± 0.011^{d}	0.919 ± 0.023^{d}	0.683 ± 0.033^{d}	F (5, 24) = 171.0

Note: DW denotes distilled water (normal control), while TE denotes testosterone enanthate (positive control).
Data are presented as mean ± SEM for each parameter, with five rats per group.
Values in the negative control group bearing a superscript (****) $p < 0.0001$ indicate a statistically significant difference when compared to the normal control group only.
Values with the superscript (d) with $p < 0.0001$ in the extract-treated groups indicate a significant difference when compared with the nicotine-only (negative control) group.

TABLE 5 | The effect of aqueous extract of *T. cistoides* on serum levels of testosterone, luteinizing hormone, and follicle-stimulating hormone.

	Control groups			*Tribulus cistoides* aqueous extract–treated groups			
Parameters	**DW 5 mL/kg**	**Nicotine 1 mg/kg**	**TE 5 + 1 mg/kg nicotine**	**50 + 1 mg/kg nicotine**	**100 + 1 mg/kg nicotine**	**150 + 1 mg/kg nicotine**	***F* (DFn, DFd)**
Serum testosterone (ng/mL)	0.521 ± 0.048	$0.124 \pm 0.002^{****}$	0.802 ± 0.060^{d}	0.283 ± 0.017^{a}	0.312 ± 0.029^{b}	0.411 ± 0.016^{d}	$F\ (5, 24) = 47.70$
Serum LH (mIU/mL)	9.518 ± 0.619	$4.057 \pm 0.343^{****}$	3.867 ± 0.229^{ns}	7.586 ± 0.649^{b}	10.450 ± 0.361^{d}	11.660 ± 0.992^{d}	$F\ (5, 24) = 31.39$
Serum FSH (mIU/mL)	1.535 ± 0.133	$0.425 \pm 0.040^{****}$	0.715 ± 0.023^{ns}	1.179 ± 0.037^{d}	1.170 ± 0.021^{d}	1.516 ± 0.118^{d}	$F\ (5, 24) = 33.18$

Note: DW denotes distilled water (normal control), while TE denotes testosterone enanthate (positive control).
Data are presented as mean ± SEM for each parameter, with five rats per group.
Values in the negative control group bearing a superscript (****) $p < 0.0001$ indicate a statistically significant difference when compared to the normal control group only.
Values with the superscript (d) indicate a significant difference with $p < 0.0001$ compared with the nicotine-only (negative control) group, while superscript (ns) denotes no significant difference relative to the normal control.

increased seminal vesicle fructose levels ($p < 0.01$) compared with the negative control.

Nicotine treatment markedly reduced testicular protein concentration (0.132 ± 0.011 mg/g vs. 0.595 ± 0.020 mg/g) relative to the normal control. Co-treatment with *T. cistoides* aqueous extract at 50, 100, and 150 mg/kg significantly elevated testicular protein levels ($p < 0.0001$) in a dose-independent manner, with the 100 mg/kg dose showing the highest increase (0.919 ± 0.023 mg/g vs. 0.132 ± 0.011 mg/g). Similarly, testosterone administration significantly increased testicular protein content compared with the nicotine-only group.

3.6 | Effect of *T. cistoides* on the Serum Concentration of Testosterone, LH, and FSH Exposed to Nicotine

As shown in Table 5, nicotine administration significantly reduced serum testosterone (0.124 ± 0.002 ng/mL vs. 0.521 ± 0.048 ng/mL), LH (4.057 ± 0.343 mIU/mL vs. 9.518 ± 0.619 mIU/mL), and FSH (0.425 ± 0.040 mIU/mL vs. 1.535 ± 0.133 mIU/mL) compared with the normal control group treated with distilled water.

Relative to the nicotine-only (negative control) group, co-administration of *T. cistoides* aqueous extract produced a dose-dependent increase in serum testosterone and LH levels, with the 150 mg/kg dose showing the greatest effect (testosterone: 0.411 ± 0.016 ng/mL; LH: 11.660 ± 0.992 mIU/mL vs. 50 mg/kg). FSH levels also increased in a dose-dependent manner, with the 50 mg/kg dose producing a moderate but significant rise (1.179 ± 0.037 mIU/mL vs. 0.425 ± 0.040 mIU/mL), while the 100 and 150 mg/kg doses caused highly significant increases (1.170 ± 0.021 mIU/mL and 1.516 ± 0.118 mIU/mL, respectively).

Administration of testosterone enanthate (5 mg/kg) alongside nicotine significantly elevated serum testosterone levels (0.802 ± 0.060 ng/mL vs. 0.124 ± 0.002 ng/mL) but did not produce a significant effect on LH or FSH compared with the nicotine-only group.

3.7 | Effect of the Aqueous Extract of *T. cistoides* on the Histological Structure of Rat Testis of Rats Exposed to Nicotine

Figure 2 shows the cross-sections of the testis of the experimental rats. In normal animals treated with distilled water, the seminiferous tubule lumen (STL) shows male sex cells (spermatogonia) at different stages of development from the periphery to the lumen. The negative controls show histopathological alterations of the testis marked by clarification of the seminiferous epithelium (CSE) and a decrease in sperm density in the STL, which has been corrected by the different doses of extract prominent with 150 mg/kg of *T. cistoides*.

4 | Discussion

Previous studies have demonstrated that several medicinal plant extracts possess protective pharmacological properties against nicotine-induced toxicities, including reproductive dysfunction [10, 11, 31]. Building on this evidence, the present study evaluated the protective and restorative effects of aqueous *T. cistoides*

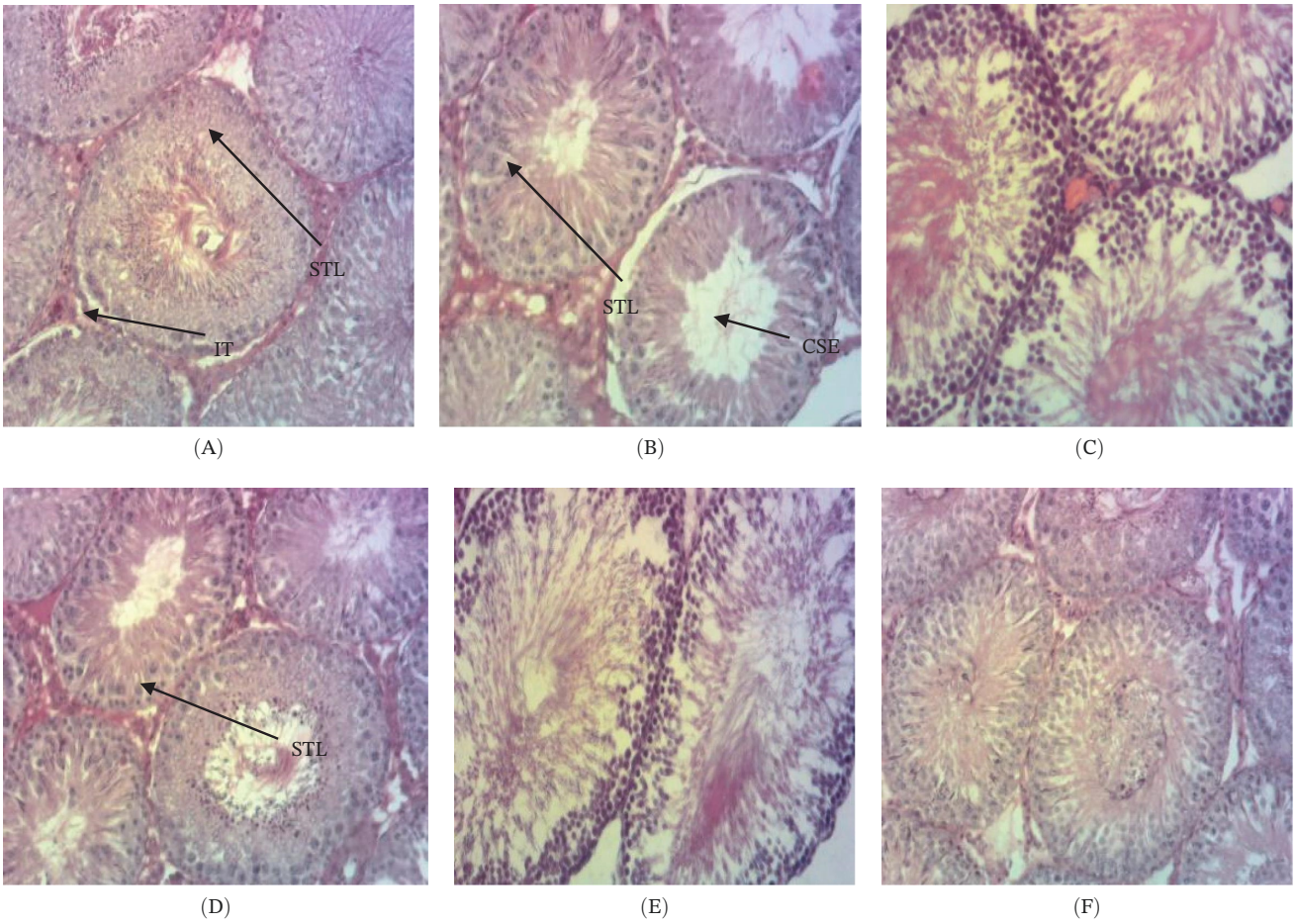


FIGURE 2 | Microphotographs showing the effect of *T. cistoides* on the histological structure of rat testis: (A) normal control, (B) negative control, (C) positive control, (D) *T. cistoides* 50 mg/kg, (E) *T. cistoides* 100 mg/kg, and (F) *T. cistoides* 150 mg/kg. CSE, clarification of seminiferous epithelium; IT, interstitial tissue; STL, seminiferous tubule lumen.

extract on nicotine-induced male infertility in Wistar rats. Nicotine was administered at 1 mg/kg for 60 days, either alone or in combination with graded doses of *T. cistoides* extract (50, 100, and 150 mg/kg), while a testosterone enanthate-treated group (5 mg/kg) served as a reference control for androgenic activity.

Body weight assessment showed no significant differences between the extract-treated groups and the nicotine-only group, although a general increase in body weight was observed across all groups, consistent with normal physiological growth. This finding indicates that *T. cistoides* extract did not adversely affect systemic metabolism or general health status, suggesting good tolerability of the extract at the administered doses. Similar observations were reported by Tangu et al. [22], who demonstrated that *G. boninense* protected male rats against reproductive and hepatic toxicity without significantly altering body weight.

Assessment of reproductive organ weights revealed that coadministration of *T. cistoides* with nicotine significantly improved testicular and accessory sex organ weights compared with the nicotine-only group. Androgens, particularly testosterone, play a crucial role in the development, maintenance, and functional integrity of male reproductive organs [32]. The observed increase in organ weights in extract-treated rats is consistent with the concomitant elevation in serum testosterone levels, which are essential for tissue growth, differentiation, and maintenance of reproductive structures [33]. Testosterone acts via androgen receptor-mediated gene activation, promoting cellular hypertrophy and protein synthesis within reproductive tissues. Histological analysis further supported these findings, as extract-treated groups exhibited increased sperm density and preserved seminiferous tubule architecture. In contrast, nicotine-treated rats showed thinning of the seminiferous epithelium and reduced sperm density, which are characteristic features of testicular damage and gonadotoxicity [34]. These alterations reflect nicotine's capacity to induce oxidative stress and disrupt testicular integrity. Comparable testicular atrophy following nicotine exposure, and its reversal by Tribulus species, has been previously reported [31], supporting the protective role of *T. cistoides* observed in the present study.

Nicotine-induced impairment of spermatogenesis is commonly manifested by reductions in sperm density and motility [10, 11, 31, 34], findings that were clearly evident in the nicotine-only group in this study. Cotreatment with *T. cistoides* extract significantly restored these sperm parameters, indicating preservation of germinal epithelium function and sperm quality. This improvement is strongly supported by the enhanced antioxidant status observed in testicular tissue, including increased activities of SOD, CAT, reduced GSH, and NO levels, along with reduced TBARS levels. Oxidative stress is a central mechanism underlying nicotine-induced testicular damage, as excessive reactive oxygen

species overwhelm endogenous antioxidant defenses, leading to lipid peroxidation, DNA damage, germ cell apoptosis, and impaired mitochondrial function [35]. These processes ultimately compromise sperm membrane integrity and motility [36]. The dose-dependent enhancement of antioxidant enzymes, particularly at 150 mg/kg, underscores the efficacy of *T. cistoides* in mitigating oxidative damage, consistent with earlier reports [31].

Nicotine exposure is also known to disrupt the hypothalamic–pituitary–gonadal (HPG) axis by suppressing FSH and LH secretion, thereby impairing testosterone synthesis and spermatogenesis [35]. In the present study, *T. cistoides* treatment significantly increased serum levels of FSH, LH, and testosterone, indicating restoration of endocrine function. Under normal physiological conditions, GnRH stimulates pituitary secretion of FSH and LH; FSH supports Sertoli cell-mediated spermatogenesis, while LH stimulates Leydig cell testosterone production [37, 38]. The concurrent elevation of testosterone and gonadotropins observed in extract-treated rats suggests that *T. cistoides* may exert both peripheral and central modulatory effects on the HPG axis. This pattern deviates from the classical negative feedback mechanism and implies a possible stimulatory influence on the hypothalamus or pituitary. Similar hormonal responses have been reported for *Citropsis articulata* and *Mystroxylon aethiopicum*, which enhanced reproductive hormone levels in male rats [32].

In contrast, the testosterone enanthate-treated group showed reduced sperm density and motility despite elevated circulating testosterone. This effect is attributable to negative feedback inhibition of the hypothalamic–pituitary axis, leading to suppressed FSH and LH secretion, reduced intratesticular testosterone, and impaired spermatogenesis. These findings are consistent with previous reports, including [22], where testosterone enanthate was used as a positive control and produced similar outcomes.

Further evidence of improved reproductive function in *T. cistoides*-treated rats was demonstrated by significant increases in total testicular protein content and seminal vesicle fructose concentration. Testicular proteins are indicative of active spermatogenic processes, while seminal fructose provides a critical energy substrate for sperm motility and viability. The elevation of these parameters is closely linked to increased testosterone levels, which regulate accessory gland function and protein synthesis in male reproductive tissues [38]. These findings align with previous studies that associate phytochemicals such as flavonoids, saponins, phytosterols, tannins, and terpenoids with enhanced androgen production and fertility outcomes [38]. Conversely, nicotine-only rats exhibited marked reductions in testicular protein content and seminal fructose concentration, consistent with nicotine's gonadotoxic effects mediated through oxidative stress and Leydig cell dysfunction [10, 11].

Collectively, the findings of this study indicate that the aqueous extract of *T. cistoides* confers protection against nicotine-induced male infertility through multiple complementary mechanisms. These include enhancement of the testicular antioxidant defense system, restoration of endocrine balance via modulation of the HPG axis, preservation of testicular histoarchitecture, and improvement of sperm quality and accessory gland function. The combined antioxidant, androgenic, and endocrine-regulatory actions of the extract act synergistically to counteract nicotine-induced reproductive toxicity.

A limitation of the present study is the lack of detailed phytochemical characterization of the aqueous *T. cistoides* extract, which precluded identification of the specific bioactive compounds responsible for the observed effects. Nevertheless, previous studies on related Tribulus species have identified steroidal saponins, flavonoids, and phenolic compounds with established antioxidant and androgen-modulating properties. Future studies incorporating phytochemical profiling and bioactive compound isolation are therefore warranted to further elucidate the molecular mechanisms underlying the reproductive benefits of *T. cistoides*.

5 | Conclusion

Nicotine administration induced infertility in male Wistar rats, an effect that was significantly ameliorated by treatment with the aqueous extract of *T. cistoides*. The protective action of the extract appears to involve restoration of testicular antioxidant status and stimulation of male reproductive hormone production, leading to improved sperm density and motility, increased seminal vesicle fructose and total testicular protein levels, and enhanced reproductive organ weights. These findings suggest that *T. cistoides* contains bioactive constituents with fertility-protective potential. Further studies are warranted to characterize the phytochemical profile of the extract and to evaluate its effects on sperm morphology and viability for a more comprehensive understanding of its impact on male fertility.

Acknowledgments

The authors express their sincere gratitude to the Laboratory of Animal Physiology at the University of Yaoundé 1 for providing support with histological analyses. Special thanks are extended to Mr. Boubga Cliford, Mr. Veranso Jude, and Mr. Chrisantus Muchvunda for their contributions to the biochemical analyses. The authors also appreciate the technical support of Professor Oumar Mahamat, as well as the financial assistance provided by Mr. Nsah Philip and Mr. Nsah Jude.

Funding

This study did not receive any funding.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All the data used for analysis in the study are available with the corresponding author and can be provided if requested.

References

1. S. C. Esteves, R. Miyaoka, and A. Agarwal, "An Update on the Clinical Assessment of the Infertile Male," *Clinics (Sao Paulo)* 66, no. 4 (2011): 691–700.

2. M. S. Abebe, M. Afework, and Y. Abaynew, "Primary and Secondary Infertility in Africa: Systematic Review With Meta-Analysis," *Fertility Research and Practice* 6, no. 1 (2020): 20.

3. P. S. Deshpande and A. S. Gupta, "Causes and Prevalence of Factors Causing Infertility in a Public Health Facility," *Journal of Human Reproductive Sciences* 12, no. 4 (2019): 287–293.

4. A. Césaire Momo Tetsatsi, D. Alumeti Munyali, G. Romeo Bonsou Fozin, E. Ngadjui, M. Wankeu-Nya, and P. Watcho, "Semen Quality Among Men Attending Urology Services in the Dschang Health District, West Cameroon: A Retrospective Study on 379 Cases," *International Journal of Reproductive Biomedicine* 18, no. 2 (2020): 121–128.

5. World Health Organization, "1 in 6 People Globally Affected by Infertility," *Neurosciences (Riyadh)* 28, no. 3 (2023): 208-209.

6. S. W. Leslie, T. L. Soon-Sutton, and M. A. B. Khan, "Male Infertility," in *StatPearls [Internet]*, (StatPearls, Treasure Island (FL), 2024).

7. P. Sengupta, S. Dutta, I. R. Karkada, and S. V. Chinni, "Endocrinopathies and Male Infertility," *Life* 12, no. 1 (2022): 10.

8. World Health Organization, "WHO Report on the Global Tobacco Epidemic, 2019: Offer Help to Quit Tobacco Use," (World Health Organization, 2019), https://apps.who.int/iris/handle/10665/326043.

9. World Health Organization, "WHO Report on the Global Tobacco Epidemic, 2023: Protect People From Tobacco Smoke," (World Health Organization, 2023), https://www.who.int/publications/i/item/9789240077164.

10. I. P. Oyeyipo, Y. Raji, B. O. Emikpe, and A. F. Bolarinwa, "Effects of Nicotine on Sperm Characteristics and Fertility Profile in Adult Male Rats: A Possible Role of Cessation," *Journal of Reproduction and Infertility* 12, no. 3 (2011): 201–207.

11. I. P. Oyeyipo and P. J. Maartens, "*In Vitro* Effects of Nicotine on Human Spermatozoa," *Andrologia* 46, no. 8 (2014): 887–892.

12. A. Rambhatla, R. Shah, G. M. Pinggera, et al., "Pharmacological Therapies for Male Infertility," *Pharmacological Reviews* 77 (2024): 100017.

13. S. Singh, V. Nair, and Y. K. Gupta, "Evaluation of the Aphrodisiac Activity of *Tribulus terrestris* Linn. in Sexually Sluggish Male Albino Rats," *Journal of Pharmacology & Pharmacotherapeutics* 3, no. 1 (2012): 43–47.

14. M. Mbouh, S. Antoine, P. Faustin, et al., "*Momordica foetida* (Cucurbitaceae) Methanol Extract Protects Against Parastar Induced Reproductive Dysfunction and Testicular Oxidative Stress in Male Rats," *Egyptian Journal of Basic and Clinical Pharmacology* 12 (2022): 101562.

15. A. S. Kada, E. Aneng, M. Mbouh, P. F. T. Manfo, S. N. Ngimgoh, and E. A. Nantia, "Testicular Antioxidants and Testosterone Enhancing Effects of the Hydroethanolic Extract of *Rauvolfia vomitoria* (Apocynaceae) in Male Wistar Rats," *Investigational Medicinal Chemistry and Pharmacology* 4, no. 2 (2021): 54.

16. M. H. A. Da Silva, G. F. Buys-Gonçalves, J. H. Duque Reis Estrada, F. J. B. Sampaio, and D. Benchimol de Souza, "What Is the Effect of *Tribulus terrestris* on Testicular Morphology and Sperm Production? An Experimental Study in the Rat Model," *Journal of Andrology* 45, no. 1 (2024): 55–64.

17. P. Goswami, S. Damor, and U. K. Sharma, "Effect of Steroidal Extract of Tribulus Alatus on Semen Parameters of Albino Mice," *Tobacco Regulatory Science (TRS)* 7, no. 6-2 (2021): 58–62.

18. J. Gavilan, "*Tribulus cistoides* (False Puncture Vine) [Data Sheet]," (CABI Digital Library, Wallingford (2022).

19. G. Brandon, "The Uses of Plants in Healing in an Afro-Cuban Religion, Santería," *Journal of Black Studies* 22, no. 1 (1991): 55–76.

20. K. Fern, "Useful Tropical Plants," 2018, http://tropical.theferns.info/.

21. Q. W. Zhang, L. G. Lin, and W. C. Ye, "Techniques for Extraction and Isolation of Natural Products: A Comprehensive Review," *Chinese Medicine* 13, no. 1 (2018): 20.

22. N. N. Tangu, A. S. Kada, M. M. Kaghou, A. L. Njouonkou, and P. F. T. Manfo, "*Ganoderma boninense* (Ganodermataceae) Aqueous Extract Protects Against Liver and Reproductive Alterations in Parastar-Exposed Male Wistar Rats," *Asian Journal of Animal and Veterinary Advances* 17, no. 4 (2022): 146–156.

23. M. Amira, M. Sahar, H. Rania, and K. Elhady, "Competence of *Panax ginseng* on Male Fertility in Cypermethrin-Exposed Rats," *Journal of Advanced Veterinary Research* 13, no. 10 (2023): 2188–2194.

24. S. B. Budin, J. H. Kho, and J. H. Lee, "Low-Dose Nicotine Exposure Induced the Oxidative Damage of Reproductive Organs and Altered the Sperm Characteristics of Adolescent Male Rats," *Malaysian Journal of Medical Sciences* 24, no. 6 (2017): 50–57.

25. E. F. Olasehinde, K. Takeda, and H. Sakugawa, "Development of an Analytical Method for Nitric Oxide Radical Determination in Natural Waters," *Analytical Chemistry* 81, no. 16 (2009): 6843–6850.

26. H. P. Misra and I. Fridovich, "The Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase," *Journal of Biological Chemistry* 247, no. 10 (1972): 3170–3175.

27. A. G. Gornall, G. S. Bardwill, and M. M. David, "Determination of Serum Protein by Means of the Biuret Reaction," *Journal of Biological Chemistry* 177, no. 2 (1949): 751–766.

28. World Health Organization, "WHO Laboratory Manual for the Examination and Processing of Human Semen," (World Health Organization (2021).

29. P. Watcho, F. Lih, P. B. D. Deeh, et al., "Aphrodisiac Property of Aqueous and Methanolic Extracts of *Raphia vinifera* (Arecaceae) in Sexually Experienced Male Rats," *International Journal of Reproductive Biomedicine* 17, no. 6 (2019): 413–424.

30. J. D. Bancroft and M. Gamble, *Theory and Practice of Histological Techniques*, 6th ed. (Churchill Livingstone/Elsevier, China, 2008).

31. R. Kunzle, M. D. Mueller, W. Hänggi, M. H. Birkhäuser, H. Drescher, and N. A. Bersinger, "Semen Quality of Male Smokers and Nonsmokers in Infertile Couples," *Fertility and Sterility* 79, no. 2 (2003): 287–291.

32. M. Wankeu-Nya, O. N. Djeumeni, Z. Nde, et al., "Aphrodisiac and Androgenic Effects of the Aqueous Extract of the Roots of *Vepris afzelii* on Cyproterone Acetate-Induced Hypogonadism in Rats," *International Journal of Impotence Research* 37, no. 4 (2025): 293–302.

33. J. Ndukui, M. Kamatenesi, O. David, and W. Paul, "Effects of Crude Aqueous Leaf Extracts of *Citropsis articulata* and *Mystroxylon aethiopicum* on Sex Hormone Levels in Male Albino Rats," *International Journal of Pharmaceutical Science Invention* 3, no. 10 (2014): 1–7.

34. D. Ray, A. Bhattercharjee, B. Majhi, and S. Mukherjee, "Amelioration of Nicotine-Induced Damages by Vitamin B_{12} and Folic Acid," *Journal of Biomedical* 39, no. 2 (2019): 353–368.

35. D. Ray and S. Mukherjee, "The Protective Effects of *Chlorophytum borivilianum* on Nicotine-Induced Reproductive Toxicity, Oxidative Damage, Histological Changes, and Hematotoxicity in Male Rats," *Journal of Integrative Pharmacy and Biological Sciences* 5, no. 2 (2018): 76–81.

36. D. Ray, "Effects of Nicotine on Sperm Health and Semen Quality: A Review Based on Animal Studies," *Journal of Drug Delivery and Therapeutics* 9, no. 4-A (2019): 754–758.

37. P. Marques, A. de Sousa Lages, and K. Skorupskaite, "Physiology of GnRH and Gonadotrophin Secretion," in *Endotext*, eds. K. R. Feingold, S. F. Ahmed, and B. Anawalt, (MDText.com, Inc, Portland, 2024, https://www.ncbi.nlm.nih.gov/books/NBK279070/.

38. M. J. T. Nangap, R. Fokou, L. Walbadet, et al., "Aphrodisiac Potentials of the Aqueous Extract of *Hibiscus asper* Hook. f. Leaves (Malvaceae) in Male Wistar Rats," *Journal of Phytopharmacology* 12, no. 1 (2023): 11–19.